

CLAIMS

1. Isolated nucleotide sequence comprising the nucleic sequence SEQ ID No. 1 or the nucleic sequence
5 SEQ ID No. 2, their complementary sequences, the fragments and derived sequences thereof, differing by mutation, insertion, deletion and/or substitution of one or more bases and hybridizing under high stringency conditions with the sequences SEQ ID No. 1 and
10 SEQ ID No. 2, respectively.
2. Isolated nucleotide sequence comprising the sequence SEQ ID No. 1, the sequences complementary thereto and the sequences derived therefrom, comprising a nucleotide chain resulting from the stable
15 combination of at least a portion of the insertion sequence IS91 and at least a portion of the sequence of the *katP* gene.
3. Isolated nucleotide sequence according to Claim 2, comprising at least 8, advantageously 10, preferably
20 14 consecutive nucleotides of the chain of the sequence SEQ ID No. 1, including the nucleotides from position 400 to 407.
4. Isolated nucleotide sequence comprising at least 8 consecutive nucleotides of the sequence
25 SEQ ID No. 1 or of the sequence SEQ ID No. 2, or of sequences complementary thereto and derived therefrom, as defined in Claim 1.
5. Isolated nucleotide sequence according to Claim 4,
30 selected from the following nucleic sequences :
- SEQ ID No. 3: 5' - CGGAGATGAAAGCACCCTGTG - 3'
- SEQ ID No. 4: 5' - GGGCTGTGTAATCTCAGAGGAG - 3'
- SEQ ID No. 5: 5' - GTCCGGAGATGAAAGCACCCTGTG - 3'
- SEQ ID No. 6: 5' - TCAGGGCTGTGTAATCTCAGAGGAG - 3'
- SEQ ID No. 7: 5' - GGCGCTGATACCGGCAAGAATGG - 3'

SEQ ID No. 8 : 5' - GGTCCCGCAGGCCATGATTTTTG - 3'
SEQ ID No. 9 : 5' - CCGGCAAGAATGGTCGCAAACCTCC - 3'
SEQ ID No. 10 : 5' - AAGGGGTTCCAAGCCGCAACTGACGA - 3'
SEQ ID No. 11 : 5' - TAAGGGGTTCCAAGCCGCAACTGACG - 3'
SEQ ID No. 12 : 5' - CTCAACGGCATCGTCAGTTGCGGCTTGGAAC - 3'
SEQ ID No. 13 : 5' - AGCACTCAACGGCATCGTCAGTTGCGGCTTG - 3'
SEQ ID No. 14 : 5' - CTATTTCAAGGATACCCTTCGTCATCAACACG - 3'
SEQ ID No. 15 : 5' - AATTTCCCTTAATCCGGAGCTATTCGTATGA - 3'
SEQ ID No. 16 : 5' - GAAGACCAGCTTTTTGTTTC - 3'
SEQ ID No. 17 : 5' - TGTCACAGACTCAATGACTA - 3'
SEQ ID No. 18 : 5' - GGCATCGTCAGTTG - 3'
SEQ ID No. 19 : 5' - CGGCATCGTCAGTTGC - 3'
SEQ ID No. 20 : 5' - ACGGCATCGTCAGTTGCG - 3'
SEQ ID No. 21 : 5' - CCACCTGAACGATAAGCGGAAC - 3'
SEQ ID No. 22 : 5' - CACCTTCCTTCCATCCTCAGAC - 3'
SEQ ID No. 23 : 5' - ATCCCAGCGCGCTCCAGCTG - 3'
SEQ ID No. 24 : 5' - ACCCATGATGGCGCATCTGATG - 3'
SEQ ID No. 25 : 5' - ACGTTCTGGTCTTACGGGTGATGTAGGTTTT - 3'
SEQ ID No. 26 : 5' - TAGTGAAGCGGTGACAGCATATCAGACGGCT - 3'
SEQ ID No. 27 : 5' - GTGAGATAGGCACAACAATGA - 3'

6. Pairs of isolated nucleotide sequences according to Claim 4 or 5, used as primers, selected from the following pairs of the following sequences :

SEQ ID No. 3 and SEQ ID No. 4
SEQ ID No. 5 and SEQ ID No. 6
SEQ ID No. 6 and SEQ ID No. 7
SEQ ID No. 6 and SEQ ID No. 8
SEQ ID No. 6 and SEQ ID No. 9
SEQ ID No. 21 and SEQ ID No. 22
SEQ ID No. 23 and SEQ ID No. 24

7. Isolated nucleotide sequence according to Claim 4 or 5, used as probe, selecting from the following

sequences: SEQ ID No. 14, SEQ ID No. 25, SEQ ID No. 15, SEQ ID No. 26, SEQ ID No. 18, and SEQ ID No. 27.

8. Isolated nucleotide sequence according to Claim 7, characterized in that it is labelled.

5 9. Isolated nucleotide sequence according to Claim 7, characterized in that it is immobilized on a support.

10. Plasmids pDF3 and pDF4 deposited at the Collection Nationale de Cultures de Microorganismes respectively under the numbers I-1999 and I-2000, on 26 March 1998.

11. Host cell comprising a plasmid according to Claim 10.

12. Method for the detection of *E. coli* O157 :H7 or EHECs in a sample, comprising the following steps:

15 (a) bringing the sample into contact with a pair of oligonucleotide primers chosen from the oligonucleotides defined in Claim 5; the nucleic acid contained in the sample having been, where appropriate, made accessible to the hybridization of the primers with the nucleic acid of the target tested for,

20 (b) amplifying the nucleic sequence flanked by the pair of primers chosen,

(c) verification of the presence of the amplified product by the use of at least one probe specific for the amplified product.

25 13. Method according to Claim 12, according to which step (c) comprises the following substeps:

30 (c₁) denaturation of the amplified sequences by a physical or chemical means,

(c₂) bringing a solution containing the denatured amplified fragments of step (c₁) into contact with, on the one hand, at least one capture probe, and on the other hand, at least one detection probe, optionally labelled, the capture and detection probes having a sequence as defined in Claim 1, and capable of hybridizing with the same strand of the amplified fragments, the said bringing into contact being

performed for a period sufficient to allow the hybridization reaction,

(c₃) at least one washing in order to remove the unreacted nucleic sequences,

5 (c₄) visualization of the detection probes hybridized with the amplified nucleic sequences.

14. Method according to Claim 12 or 13, in which the capture probe is attached to the surface of a well of a microtitration plate.

10 15. Method according to Claim 12 or 13, in which the detection probe is labelled with peroxidase.

16. Method according to any one of Claims 13 to 15, characterized in that the detection of the activity of the peroxidase linked to the detection probe which has reacted, is carried out by colorimetric reaction, in the presence of a chromogenic substrate, such as tetramethylbenzidine (TMB), using the following steps:

15 - addition of the chromogenic substrate, such as a TMB solution, to the wells containing the reaction mixture,

20 - incubation, in the dark, for a sufficient period to allow the colour to develop,

- blocking of the reaction by addition of a blocking solution,

25 - determination of the optical density at an appropriate wavelength.

17. Method for the detection of *E. coli* O157 :H7, according to any one of Claims 12 to 16, using the following oligonucleotides:

30 - the sequences SEQ ID No. 5 and SEQ ID No. 6, as primers for the amplification,

- the sequence SEQ ID No. 15, as capture probe,

- the sequence SEQ ID No. 18 as detection probe.

35 18. Method for the detection of the EHECs, according to any one of Claims 12 to 16, using the following oligonucleotides:

- the sequences SEQ ID No. 21 and SEQ ID No. 22, as primers for the amplification,

- 31 -

- the sequence SEQ ID No. 25, as capture probe,
- the sequence SEQ ID No. 27, as detection probe.

19. Kit for the detection of *E. coli* O157 :H7 or
5 EHECs, comprising among the reagents:

- at least two oligonucleotides according to Claim 5, used as a pair of primers,
- optionally at least one oligonucleotide probe according to Claim 5, for the detection of the
10 amplified product.